

**Amendments to the specification:**

*Rewrite the paragraph bridging pages 1 and 2:*

Schneewind et al. (Cell, Vol. 70, p. 267-281, 1992) have studied the anchoring mechanism of protein A in the cell wall of *Staphylococci*. Protein A belongs to a growing class of surface proteins of Gram-positive bacteria which are characterized by a succession of the characteristic sequence motif LPXTG (SEQ ID NO: 11), followed by a group of 15-22 hydrophobic amino acids, and a C-terminal group of 5-12 charged amino acids. The conservation of these elements is considered an indication of a common export mechanism of these proteins in different Gram-positive species. In order to establish the localization of protein A (discrimination between protein A anchored in the cell wall and secreted protein A) in *S. aureus*, the authors employ radioactive labeling methods. The importance of the above mentioned sequence elements to cell wall anchoring is supported by using hybrid proteins and through mutagenesis of the LPTXG (SEQ ID NO: 11) motif and of the C terminus. However, Schneewind et al. are concerned neither with enzymes which might catalyze the anchoring of the surface proteins, nor with their inhibition.

*Rewrite page 2, 1<sup>st</sup> complete paragraph:*

The cell wall anchoring elements in surface proteins of Gram-positive bacteria are also the subject of another article by Schneewind et al. (EMBO J., Vol. 12, p. 4803-4811, 1993). It is shown that enterotoxin B, a protein normally secreted into the medium, can be anchored in the *Staphylococcus*

cell wall through C terminal fusion to the protein A anchoring signal. The results support the hypothesis that the cell wall sorting is accompanied by a proteolytic cleavage of the polypeptide chain at the C-terminus. Presumably, the LPXTG (SEQ ID NO: 11) motif is the site of such cleavage and covalent binding to the cell wall while the charged sequence segment serves as a retention signal during the cell wall sorting. The relevance of the geometrical length of the hydrophobic domain, which is dependent on the folding properties, is confirmed by the experiments.

*Rewrite page 3, 1<sup>st</sup> complete paragraph:*

The structure of the cell wall anchor of the surface proteins in *Staphylococcus aureus* is the subject of a report by Schneewind et al. (Science, Vol. 268, p. 103-106, 1995). The authors use a combination of a molecular-biological and mass-spectrometric technique and are able to show that after cleavage of the surface protein between threonine and glycine of the concerved LPXTG (SEQ ID NO: 11) motif, the carboxy group of threonine is covalently bonded via transpeptidization to the murein sacculus with the free amino group of the cell pentaglycine. However, Schneewind et al. also fail to identify or characterize the protein believed to be responsible for proteolysis and transpeptidization, the so-called sortase.

*Rewrite page 5, last paragraph:*

In a preferred embodiment, the method according the invention is to be considered an enzymatic reporter assay which detects the effect of substances an bacterial factors (targets ) which directly or

indirectly participate in the LPXTG (SEQ ID NO: 11)-motif-dependent C-terminal anchoring of polypeptides to the surface of Gram-positive bacteria. Among the large number of factors and processes which may have an effect on this process, the present invention preferably aims at those enzymatic steps which take place after the beginning of the translocation of the cellular surface polypeptides over the cytoplasmic membrane. In addition, the method according to the invention in part covers enzymatic and other targets which participate in the biosynthesis of cell wall murein. From the phenotypical characteristics of the cells used in the respective method, potentially active substances can be assigned to particular groups of targets.

*Rewrite page 6, paragraph 1:*

In particular preferred embodiment of the method according to the invention, the cellular and molecular basis of the reporter assay is a recombinant *Staphylococcus carnosus* clone which contains a selectable expression plasmid with an inducible reporter gene fusion. The gene fusion codes for a hybrid polypeptide consisting of N-terminal signal peptide, a precursor protein of *Staphylococcus hyicus* lipase and a C-terminal portion of the fibronectin binding protein B (FnBPB) from *Staphylococcus aureus*. After being produced in the cytoplasm, the hybrid polypeptide is transported through the bacterial cell membrane due to its N-terminal signal structures, and processed at the amino terminus by a signal peptidase. Further, a cleavage in the C-terminal LPXTG (SEQ ID NO: 11) recognition motif is performed, the remaining hybrid protein is covalently linked to the murein. It has been experimentally established that different lengths of the FnBPB portion influence the

building of enzymatic activity of the lipase fusions differently. One construct was identified which exhibited no lipase activity in its cellular-surface bound form (coded by plasmid pTX30Δ82). However, if the corresponding fusion was released from the bacterial surface by treatment with lysostaphin after having been covalently anchored, the full lipase activity was achieved. Within the scope of the present invention, it has been recognized for the first time that interferences with the cell wall anchoring of the lipase function result in a release of the fusion with a concomitant occurrence of lipase activity in the culture supernatants in the assay clone in question. As possible targets, various cellular factors may be considered which can be essentially divided into two groups according to their growth behavior.

*Rewrite the paragraph bridging pages 6-7:*

One target or group of targets is the enzyme or enzyme complex designated as sortase which effects the carboxy-terminal cleavage of relevant polypeptides in the LPXTG (SEQ ID NO: 11) motif and their subsequent covalent bonding to peptide components of the cell wall murein, such as interpeptide bridges, especially pentaglycine units, on the surface of the bacteria. Although the inhibition of these functions presumably leads to the release of surface-bound factors and thus probably to the attenuation of impairments of the viability and dividability of the bacteria. A characteristic phenotypical feature of this group of targets in the assay procedure is the release of lipase activity into the assay medium while the growth behavior of the bacteria is more or less unaffected. In contrast, the impairment of a target or group of targets having an essential function

in murein synthesis results in massive changes in the viability and dividability of the bacteria which are phenotypically detectable.

*Rewrite the paragraph bridging pages 7-8:*

After induction of the xylose promoter *S. carnosus/pTX30Δ82 . mem* produces a hybrid protein consisting of *S. hyicus* propilase and the C-terminal fragment of *S. aureus* fibronectin binding protein B (FnBPB) wherein the LPXTG (SEQ ID NO: 11) motif, which is important to the anchoring to the cell wall, has been exchanged for the sequence ISQAS (Figure 1). In other embodiments, however, it may also be exchanged for any other sequence which preferably consists of 5 amino acids. In a further embodiment, LPXTG (SEQ ID NO: 11) substitution is not necessary for the mem phenotype, but a complete deletion of the LPXTG (SEQ ID NO: 11) motif may also be considered. Thus, the specific cleavage at the LPXTG (SEQ ID NO: 11) motif and hence the covalent binding to the murein sacculus do not take place. Surprisingly, it could be shown that the hydrophobic anchoring sequence which is thus retained at the carboxy terminus of the fusion is evidently not sufficient to anchor the lipase stably and especially in an inactive form in the cell coat and thus at the cellular surface. The lipase activity is quantitatively released from the bacterial surface into the culture medium. Thus, this clone simulates the inhibition of the cleavage reaction by sortase, an essential step which preceeds the covalent bonding of the N-terminal cleavage product to the murein at the cell wall.

*Rewrite page 9, paragraph 4:*

The method according to the invention advantageously allows the selective identification of such active substances as directly or indirectly affect the covalent bonding of polypeptides to the surface of Gram-positive bacteria. As set forth above, the putative process of surface anchoring comprises two specific steps:

- a) cleavage between threonine and glycine of the LPXTG (SEQ ID NO: 11) motif; and
- b) covalent bonding of the threonine to peptide components of the cell wall, especially the interpeptide bridge.

*Rewrite the paragraph bridging pages 10-11:*

In a preferred embodiment of the method according to the invention, a hybrid polypeptide having a succession, in particular, of the following sequence segments is used as a reporter substance: N-terminal signal peptide, enzyme, sequence segment having the sequence having the sequence LPXTG (SEQ ID NO: 11), hydrophobic sequence segment, and charged sequence segment. The signal peptide is proteolytically removed in the course of the secretory processing pathway. As shown in the following Examples, *S. hyicus* lipase, in particular, can be used as the enzymatic component of the hybrid polypeptide. However, it may also be of advantage to use *E. coli*  $\beta$ -lactamase or other enzymes. Naturally occurring surface polypeptides as well as genetically engineered hybrid polypeptides may serve as reporter substances which can be detected in the medium due to the action of suitable active substances, using the whole range of known chemical,

biochemical and immunological methods. However, in order to ensure a high sample throughput while maintaining the reliability of the method according to the invention, it is advantageous, in particular, to use hybrid polypeptides having enzymatic activity as reporter substances. Thus, those active substances which affect the proteolytic cleavage of the signal peptide as well as the whole transport route through the cytoplasmic membrane do not result in the appearance of the active reporter substance in the supernatant.

*Rewrite page 11, 3<sup>rd</sup> complete paragraph:*

In addition, it may be particularly preferred to determine the change in enzymatic activity due to a transition of the enzyme from an inactive to an active conformation or vice versa. This may be preferably achieved by using a linker peptide provided between the enzyme and the LPXTG (SEQ ID NO: 11) motif.

*Rewrite page 11, 4<sup>th</sup> complete paragraph:*

In a preferred embodiment of the method according to the invention, the number of amino acids of the linker peptide is chosen such that enzyme is anchored to the surface of the Gram-positive bacteria in an inactive conformation. When *S. hyicus* lipase is used, the number of amino acids in the linker peptide should be less than ten, in particular. In another embodiment, the enzyme could be directly fused with its C terminus to the LPXTG (SEQ ID NO: 11) motif, avoiding the linker peptide. Thus, in the absence of the active substance, the Gram-positive bacteria bear inactive enzymes covalently

bonded to their surface which, when not covalently bonded, especially if released from the surface of the Gram-positive bacteria, fold into an active conformation and thus undergo a detectable change in one of their properties, i.e., enzymatic activity in this case.